

WHAT WE CLAIM IS

1. A recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from
 - 5 (a) the sequence of SEQ ID No:1;
 - (b) a functionally equivalent variant of the sequence of SEQ ID NO:1 which has greater than 77% amino acid sequence identity with SEQ ID NO:1; and
 - (c) a functionally equivalent fragment of a polypeptide defined in (a) or
- 10 (b).
2. A recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from
 - (a) amino acids 20 to 235 of SEQ ID NO:1
 - (b) a functionally equivalent variant which has greater than 77% amino
 - 15 acid sequence identity with amino acids 20 to 235 of SEQ ID NO:1; and
 - (c) a functionally equivalent fragment of a polypeptide defined in (a) or
- (b).
3. A polypeptide as claimed in claim 2 wherein the sequence has greater than
- 20 90% identity with SEQ ID NO:1.
4. A polypeptide as claimed in claim 2 wherein the sequence has greater than 99% identity with the sequence of amino acids 20 to 235 of SEQ ID NO:1.
5. A polypeptide as claimed in claim 2 wherein the sequence is that of amino acids 20 to 235 of SEQ ID NO:1.
- 25 6. A polypeptide as claimed in any one of claims 1 to 5 which is obtainable from a bacterium.
7. A polypeptide as claimed in any one of claims 1 to 5 which is obtainable from *Mycobacterium avium* subspecies *paratuberculosis*.
8. A polypeptide as claimed in any one of claims 1 to 5 which is obtainable
- 30 from a heterologous host transformed with a polynucleotide which encodes

said polypeptide or functionally equivalent variant or fragment thereof wherein said host is capable of expressing said polypeptide.

9. A polypeptide as claimed in claim 8 wherein the host is *E coli*.
10. A genetic construct comprising
 - 5 (a) a promoter sequence;
 - (b) an open reading frame polynucleotide encoding a polypeptide as claimed in any one of claims 1 to 5;
 - (c) a termination sequence.
11. A recombinant, purified, or isolated polynucleotide comprising the
10 sequence of SEQ ID NO:2 or a variant thereof encoding either the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a functionally equivalent fragment of said polynucleotide.
12. A recombinant, purified or isolated polynucleotide with a nucleotide sequence complementary to the polynucleotide of claim 11.
- 15 13. One or more oligonucleotide or polynucleotide primers capable of amplifying a polynucleotide which encodes a polypeptide as claimed in claim 1 or claim 2 in a Polymerase Chain Reaction or other polynucleotide amplification method.
14. A purified or isolated antibody capable of binding a polypeptide as defined
20 in claim 4 or 5.
15. A vaccine composition comprising a polypeptide as claimed in any one of claims 1 to 8 and an acceptable diluent, carrier, excipient, or adjuvant, said polypeptide being present in an amount sufficient to generate a protective immune response to *Mycobacterium avium* subspecies *paratuberculosis*
25 infection.
16. A diagnostic composition for use in detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis*, wherein said composition comprises a polypeptide as claimed in claims 1 to 8.
17. A diagnostic composition for detecting the presence of *Mycobacterium*
30 *avium* subspecies *paratuberculosis*, wherein said composition comprises a polynucleotide according to claim 11 or claim 12.

18. A diagnostic composition for detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis* comprising at least one oligonucleotide or polynucleotide primer capable of amplifying a polynucleotide which encodes a polypeptide as claimed in any one of claims 1 to 8 in a
5 Polymerase Chain Reaction or other polynucleotide amplification method.
19. A diagnostic composition for detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis* comprising an antibody according to claim 14.
20. A method of detecting Johne's disease including preclinical Johne's disease
10 in an animal comprising contacting either the animal or a sample from the animal with a polypeptide as claimed in any one of claims 1 to 8 and detecting an immune response indicative of the presence of *Mycobacterium avium* subspecies *paratuberculosis*.
21. A method according to claim 20 wherein the response is a delayed-type
15 hypersensitivity response.
22. A method according to claim 20 wherein said detecting comprises detecting the presence of antibodies that bind a polypeptide as claimed in claim 4 or 5.
23. A method according to claim 22 wherein the detection of the presence of
20 antibodies is by ELISA, radioimmunoassay or Western blotting.
24. A method of detecting Johne's disease including preclinical Johne's disease in an animal comprising contacting a sample from the animal either with antibody according to claim 14 or a composition comprising an antibody specific to the polypeptide defined in claim 4 or claim 5 and detecting a
25 polypeptide which binds to the antibody.
25. A method according to claim 24 wherein the presence of bound antibody is determined by ELISA, radioimmunoassay or Western blotting.
26. A method according to claim 24 for detecting the presence of
30 *Mycobacterium avium* subspecies *paratuberculosis* at a preclinical phase of Johne's disease.

27. A method of detecting Johne's disease including preclinical Johne's disease in an animal comprising contacting a sample from the animal with a composition comprising of at least one oligonucleotide or polynucleotide primers capable of amplifying a polynucleotide which encodes a polypeptide as claimed in claim 4 or claim 5 in a polynucleotide amplification method and detecting the amplification product.
28. A method as claimed in claim 27 wherein the polynucleotide amplification method is a polymerase chain reaction method.
29. A method according to claim 22 for detecting the presence of *Mycobacterium avium* subspecies *paratuberculosis* at a preclinical phase of Johne's disease.
30. A method of detecting Johne's disease in an animal comprising contacting a sample from the animal with a composition comprising a polynucleotide capable of binding to a polynucleotide which encodes a polypeptide as claimed in claim 4 or claim 5.
31. A method according to claim 30 wherein said polynucleotide is detectably labeled.
32. A method according to claim 31 wherein said detectable label is a radioisotope or fluorescent tag.
33. A method of prophylactically or therapeutically treating an animal against Johne's disease which comprises administering to an animal a polypeptide as claimed in any one of claims 1 to 5 to produce a protective immunological response in the animal.
34. A method according to claim 33 which is a therapeutic method.
35. A method according to claim 33 which is a prophylactic method.
36. A method of vaccinating against Johne's disease which comprises administering to an animal a vaccine composition as claimed in claim 15 in an amount sufficient to produce a protective response.
37. A method according to claim 36 wherein said administration is performed on a single occasion.

38. A method according to claim 36 wherein said administration is performed on more than one occasion.
39. A method as claimed in claim 3 wherein 0.1-1000µg/Kg is administered of a recombinant, purified, or isolated polypeptide comprising an amino acid sequence selected from
- 5 (a) the sequence of SEQ ID No:1;
- (b) a functionally equivalent variant of the sequence of SEQ ID NO:1 which has greater than 77% amino acid sequence identity with SEQ ID NO:1; and
- 10 (c) a functionally equivalent fragment of a polypeptide defined in (a) or (b).
40. A method as claimed in claim 39 wherein 5-500µg/Kg of the polypeptide is administered.
41. A kit for use in detecting the presence of *Mycobacterium avium* subspecies
- 15 *paratuberculosis* comprising at least two of the following:
- a polypeptide as claimed in any one of claims 1 to 8;
- an antibody that binds said polypeptide, and
- a reagent for determining antigen-antibody binding.
42. A host cell transformed with a polynucleotide of claim 11 or claim 12.
- 20 43. A vector comprising the construct as claimed in claim 10.
44. A host cell incorporating a construct of claim 10.
45. A host cell incorporating a vector as claimed in claim 43.
46. A host cell according to claim 45 wherein said vector exists within the host cell as a plasmid.
- 25 47. A host cell according to claim 45 wherein said vector is integrated into the genome of the host cell.
48. A method as claimed in any one of claims 20 to 32 wherein the animal is a ruminant.
49. A method as claimed in claim 47 wherein the animal is a sheep.
- 30 50. A method as claimed in any one of claims 33 to 40 wherein the animal is a ruminant.

51. A method as claimed in claim 50 wherein the ruminant is a sheep.